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Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection

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ABSTRACT

Several procedures using high-performance liquid chromatography with photodiode-array detection have been developed to create phytochemical and toxicological profiles of phenylalkylamine derivatives in biological samples (*e.g.* plant materials and urine). Mescaline-containing cactus samples were extracted with basic methanol, using methoxamine as internal standard; the extraction and clean-up of urine samples were performed on cation-exchange solid-phase extraction columns. The extracts were separated on a 3-μm ODS column with acetonitrile–water–phosphoric acid–hexylamine as the mobile phase. Peak detection was performed at 198 or 205 nm; peak identity and homogeneity were ascertained by on-line scanning of the UV spectra from 190 to 300 nm. The detection limit of phenylalkylamine derivatives in urine and cactus material was 0.026–0.056 μg/ml and 0.04 μg/mg, respectively. Following a single oral dose of 1.7 mg/kg methylenedioxymethylamphetamine (MDMA) the concentrations found in urine ranged from 1.48 to 5.05 μg/ml MDMA and 0.07–0.90 μg/ml methylenedioxyamphetamine (a metabolite of MDMA). The mescaline content of the cactus *Trichocereus pachanoi* varied between 1.09 and 23.75 μg/mg.

INTRODUCTION

Many natural and synthetic phenylalkylamine derivatives such as mescaline, amphetamine, methylamphetamine and 4-bromo-2,5-dimethoxyphenethylamine (see Table I) are known for their stimulant and/or hallucinogenic properties. Some very active ring-substituted amphetamines such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDE) have now appeared as "designer drugs" on the illicit market, produced by clandestine laboratories [1]. Owing to their high potential of abuse, most of these popular recreational substances are now internationally controlled. Despite this, MDMA ("Ecstasy", "XTC", "Adam") is used more frequently as a controversial adjunct in psychotherapy [2,3]. *Trichocereus pachanoi* Britt. et Rose ("San Pedro") grows in subtropical and temperate areas of South America, especially in the Andean regions and belongs, together with *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult. [4], to the mescaline-containing cactus species which are commercially available without legal restrictions in Switzerland and other European countries.

Considering the potential of abuse of phenylalkylamine derivatives and mescaline-containing cactus species, it was the aim of this work to develop a selective, specific and sensitive analytical procedure using high-performance liquid chromatography with photodiode-array detection (HPLC–DAD). This method should allow not only the identification of such compounds, but also the acquisition of phytochemical and pharmacokinetic profiles in complex biological samples to estimate their toxicological or therapeutic potency. The efficiency of HPLC–DAD in drug analysis, analytical toxicology, forensic chemistry and phytochemistry of psychotropic drugs has been shown previously [5–18].

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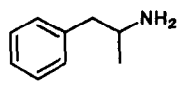
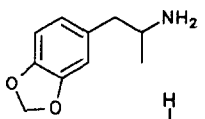
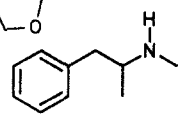
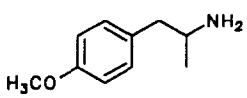
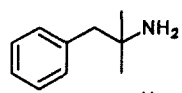
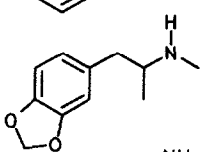
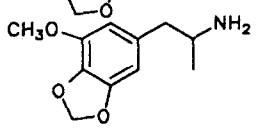
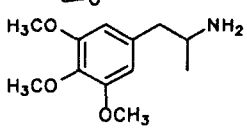
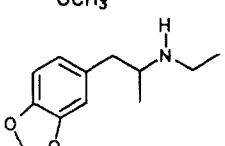
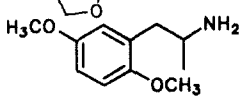
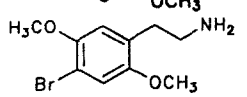
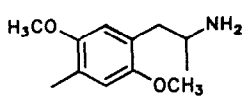
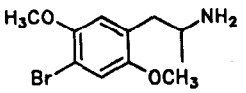
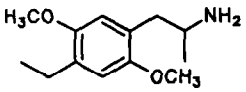
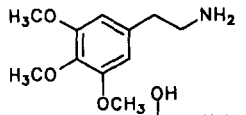
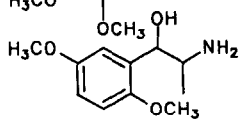
TABLE I

STRUCTURES OF PHENYLALKYLAMINE DERIVATIVES

Structure	Peak no.	Compound
	1	Amphetamine
	2	3,4-Methylenedioxyamphetamine (MDA)
	3	Methylamphetamine
	4	4-Methoxyamphetamine
	5	Phentermine
	6	3,4-Methylenedioxymethamphetamine (MDMA)
	7	5-Methoxy-3,4-methylenedioxyamphetamine (MMDA)
	8	3,4,5-Trimethoxyamphetamine
	9	3,4-Methylenedioxyethylamphetamine (MDE)
	10	2,5-Dimethoxyamphetamine
	11	4-Bromo-2,5-dimethoxyphenylethylamine (DOBP,2-CB)
	12	2,5-Dimethoxy-4-methylamphetamine (DOM,STP)

(*Continued on p. 89*)

TABLE I (*continued*)

Structure	Peak No.	Compound
	13	4-Bromo-2,5-dimethoxyamphetamine (DOB)
	14	2,5-Dimethoxy-4-ethylamphetamine (DOET)
	15	Mescaline
	16	Methoxamine

EXPERIMENTAL

Instrumentation

The HPLC–DAD system consisted of a Hewlett-Packard 1090M liquid chromatograph (Hewlett-Packard, Waldbronn, Germany), an HP 1090L autosampler, an HP 1040M photodiode-array detector, an HP 79994A Chemstation (software version 1.05), an HP 7470A *x/y* plotter and an HP 2225A Thinkjet printer.

Chromatographic conditions

The separation of fourteen phenylalkylamine derivatives was performed at 40°C on a 125 × 4.0 mm I.D. column packed with 3-μm Spherisorb ODS-1 (Phase Separations), filled by Stagroma (Wallisellen, Switzerland). The solvent gradient was developed by using the CARTAGO (computer assisted retention time prediction and gradient optimization) software; details of this procedure are published elsewhere [19,20]. Solvent A was water containing 5.0 ml (8.5 g) orthophosphoric acid (85%) and 0.28 ml (0.22 g) hexylamine per 1000 ml; solvent B was acetonitrile containing 100 ml water, 5.0 ml (8.5 g) orthophosphoric acid (85%) and 0.28 ml (0.22 g) hexylamine per 1000 ml. The gradient profile was as follows: 0–10.6 min, 5.5% B in A (isocratic); 10.6–21.6 min, 5.5–39% B in A (linear gradient). The flow-rate was 0.8 ml/min. The eluent was filtered through a membrane filter (regenerated cellulose, 0.45 μm, Schleicher and Schuell) and degassed by sonication and during use with a constant flow of helium. Methanol was used for washing the column.

The separation of urine samples containing MDMA and MDMA metabolites was performed isocratically at 40°C on a 125 × 4.0 I.D. column packed with 3-μm Spherisorb ODS-1. The mobile phase was acetonitrile–water (72:928, v/v; 57:943, w/w), containing 5.0 ml (8.5 g) orthophosphoric acid (85%) and 0.28 ml (0.22 g) hexylamine per 1000 ml. The flow-rate was 0.8 ml/min.

The separation of the cactus samples was performed isocratically at 25°C on a 150 × 4.6 mm I.D. column with a 20 × 4.0 mm I.D. precolumn, packed with 3-μm Spherisorb ODS-1. The mobile phase was acetonitrile–water (108:892, v/v), containing 5.0 ml (8.5 g) orthophosphoric acid (85%) and 0.28 ml (0.22 g) hexylamine per 1000 ml. The flow-rate was 1 ml/min.

Chemicals and reagents

Amphetamine sulphate was obtained from Siegfried (Zofingen, Switzerland) and methylamphetamine hydrochloride from Dr. Grogg Chemie (Berne, Switzerland). Mescaline hydrochloride was supplied by Laboratoires Plan (Geneva, Switzerland), methoxamine hydrochloride and MDA were

provided by Sigma (St. Louis, MO, USA). MDMA, MDE, 5-methoxy-3,4-methylenedioxyamphetamine (MMDA), 4-methoxyamphetamine, 2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-methylamphetamine (DOM,STP), 2,5-dimethoxy-4-ethylamphetamine (DOET), 3,4,5-trimethoxyamphetamine, 4-bromo-2,5-dimethoxyamphetamine (DOB) and phentermine were donated by the Division of Narcotic Drugs, United Nations (Vienna, Austria). 4-Bromo-2,5-dimethoxyphenethylamine (DOBP,2-CB) was a gift of the Swiss Association for Psycholytic Therapy. All other chemicals and reagents were of HPLC or analytical-reagent grade and were purchased from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland).

Urine and cactus samples

The urine samples were obtained from patients treated with MDMA by psychiatrists of the Swiss Association for Psycholytic Therapy. Urine samples were collected approximately 6 h after the administration of 1.7 mg/kg MDMA. The specimens of *Trichocereus pachanoi* Britt. et Rose and *Lophophora diffusa* (Croizat) Bravo (Cactaceae) were bought at flower shops and shopping centres in Switzerland, or obtained from private collections.

Sample preparation

The extraction and clean-up of urine samples (real, spiked, blank) were carried out on Adsorbex SCX (100 mg) cation-exchange extraction columns (Merck), using an Adsorbex SPU sample preparation unit. Frozen urine samples (stored at −20°C) were warmed to room temperature in an ultrasonic bath and centrifuged, if necessary (2000 *g* for 5 min). An aliquot of 1.0 ml was added to 0.5 ml of 0.05 *M* KH_2PO_4 and then sonicated for 1 min in a stoppered 2.5-ml vial. The Adsorbex columns were preconditioned with 2 ml of methanol, 1 ml of water and 1 ml of 0.017 *M* KH_2PO_4. The sample was then applied to the preconditioned extraction column, which was not allowed to dry out at the end of the the preconditioning step. The vial was rinsed with 0.05 ml of 0.05 *M* KH_2PO_4. After drying the extraction column for about 1 min, urine interferences were removed by washing the cartridge with 3 × 0.5 ml of 0.017 *M* KH_2PO_4 and 1 ml of methanol, followed by drying the column for about 1 min under vacuum. The elution step was carried out with 4 × 0.5 ml of methanol–hydrochloric acid (7.3%; 97.5:2.5) at a flow-rate of about 0.5 ml/min. Aliquots of 10 µl of the defined volume of the eluates were injected into the HPLC–DAD system for the determination of MDMA and MDA. For low MDMA and MDA levels a concentration step may be necessary. An aliquot of 1.5 ml of the eluates was added to 68 µl of 1 *M* K_2HPO_4, concentrated to about 100 µl under a stream of nitrogen and reconstituted to 150.0 µl with methanol–water (50:50). The resulting solution, with a pH of about 4–5, was then sonicated for 2 min and filtered through the tip of a Pasteur pipette filled with cotton wool. Aliquots of 10 µl were used for determination by HPLC–DAD.

The cactus samples were cut in half, lyophilized and stored in a desiccator under vacuum and protected from light until used for analysis. A representative sample of the cactus specimen was pulverized with a grinder. An accurately weighed amount of the powdered sample (about 10 mg) was then washed four times with 1 ml of diethyl ether by sonication (5 min) and filtration through a 0.2-µm regenerated cellulose filter (Spartan 13/30, Schleicher & Schuell). The defatted sample was extracted four times with 0.5 ml of methanol–ammonia (33%; 99:1), containing 150.0 µg/ml methoxamine hydrochloride as internal standard (I.S.), by sonication (5 min) and filtration through a 0.2-µm regenerated cellulose filter. Aliquots of 5 µl were injected into the HPLC–DAD system.

Quantitation

Quantitation of the urine samples was performed at 198 nm by measuring the peak areas of MDMA and MDA and using the external standard method. The calibration graphs for MDMA and MDA (linear regression analysis) were obtained by analyzing twice pooled blank urine spiked with MDMA and MDA in the concentration ranges 0.5–17 and 0.08–1.6 µg/ml, respectively. The extraction was performed as described earlier. Quantitation of the cactus samples was performed by measuring the peak areas of mescaline and the I.S. at 205 nm. The calibration graph was obtained by measuring three times standard solutions in the concentration range 20–75 µg/ml mescaline with an addition of 128 µg/ml I.S. (aqueous solution, calculated as base).

Precision

The inter-day precision of MDMA in urine was determined by analyzing two pooled urine samples spiked with 1.8 and 8.8 μg/ml MDMA. The inter-day precision of MDA in urine was determined by analyzing two pooled urine samples spiked with 0.4 and 0.8 μg/ml MDA. The intra-day precision of mescaline in the cactus material was determined by analyzing a dried and pulverized mescaline-free cactus specimen (*Lophophora diffusa*) spiked with a methanolic solution of 1 mg/ml mescaline (corresponding to 10 μg/mg dried material). The solvent was evaporated before analysis. All analyses were repeated three times on two different days during a one-week period using the described procedures.

Recovery study

The recoveries of MDMA and MDA from urine specimens were measured with the spiked samples used for the determination of the precision. The recovery of mescaline from cactus material was determined with a dried and pulverized mescaline-free cactus sample (*Lophophora diffusa*) spiked with a solution of 1 mg/ml mescaline (corresponding to 10 μg/mg dried material). The efficiency of the extraction and clean-up procedures was calculated by comparing the peak areas of MDMA, MDA and mescaline with those of similar aqueous standard solutions. All analyses were performed three times following the described procedures, but without adding I.S. for the cactus material.

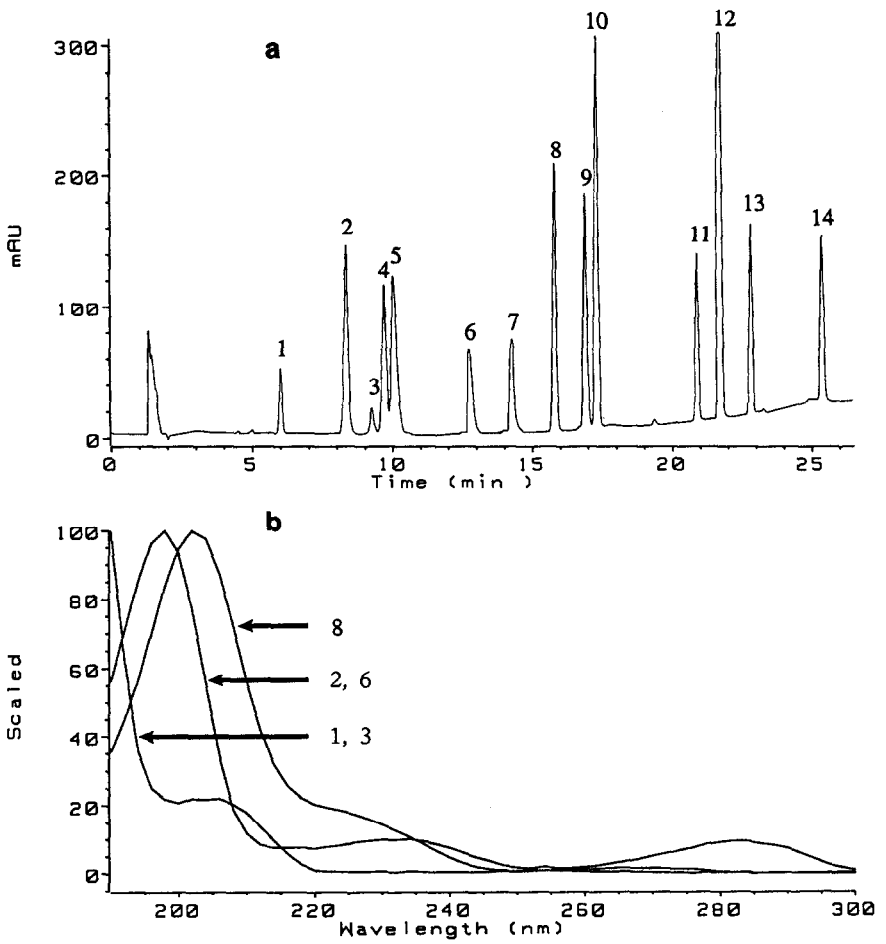


Fig. 1. (a) Chromatogram recorded at 198 nm and (b) on-line UV spectra of a standard mixture of phenylalkylamine derivatives. For peaks, see Table I. Chromatographic conditions as described under Experimental.

RESULTS AND DISCUSSION

HPLC–DAD

Among the reversed-phase materials tested (C_8, C_{18}), with particle sizes of 3, 4 and 5 μm and from different manufacturers, only the 3-μm spherical C_{18} phase with a minimum plate number of 120 000/m showed the efficiency necessary to obtain the HPLC profiles of a complex mixture of fourteen structurally related phenylalkylamine derivatives (Fig. 1a) and to produce peak shapes which were generally sharp and symmetrical. It is well known that basic compounds may show a pronounced tailing effect on certain reversed-phase columns due to interactions with the residual polar silanol groups of the stationary phase [21,22]. The addition of an amine modifier to the mobile phase as a masking agent for the silanol groups improves the peak shape and changes the capacity factor (k') of basic substances [23,24]. It has to be noted that the selectivity of the chromatographic system can be widely influenced by changing not only the ratio of acetonitrile–water but also the concentration of hexylamine. With the addition of orthophosphoric acid to the mobile phase, an acidic eluent with a pH of approximately 2 is obtained, so that the components of interest, such as MDMA, MDA, mescaline and other basic phenylalkylamine derivatives, are protonated and eluted as associates with phosphate ions. The gradient was designed by the application of the CARTAGO software, a computer-based method development tool which can be extremely useful in the selection and optimization of chromatographic conditions for complex mixtures. The details of CARTAGO are reported elsewhere [19,20].

The selectivity of HPLC is significantly improved by coupling with a photodiode-array detector, allowing peak identification through the retention time and UV spectrum as well as peak purity checks through up-slope, apex and down-slope spectra matching. Fig. 1b, with the UV spectra (190–300 nm) of amphetamine sulphate, methylamphetamine hydrochloride, MDMA, MDA and 3,4,5-trimethoxyamphetamine (see Table I) shows that only phenylalkylamine derivatives with a distinct ring-substitution pattern can be differentiated. The low UV cut-off of the water–acetonitrile modifier phase gives the possibility of measuring in a range (190–210 nm) where the phenylalkylamine derivatives exhibit the highest absorption. At the optimum detection wavelengths of 198 and 205 nm the sensitivity is approximately ten times greater for MDMA and MDA (log ε_{198} 4.557 and 4.569) and more than 60 times greater for mescaline (log ε_{205} 4.675) compared with the absorption maxima and detection wavelengths reported previously (233–234 nm for MDMA and MDA [25,26] and 268 nm for mescaline [27]). The detection limit for MDA at 198 nm and a signal-to-noise ratio of 5:1 was about 0.03 μg/ml in urine (Table II). The excellent sensitivity, the wide linearity range and the good overall reproducibility allow the detection and reliable determination of phenylalkylamine derivatives in biological matrices, even at very low concentrations.

Determination of MDMA and MDA in urine

The use of solid-phase extraction as an alternative to liquid–liquid extraction for the isolation of xenobiotics from body fluids is recommended because of excellent recoveries and ease of operation [5,18]. The sample clean-up of small volumes of urine containing MDMA and its main metabolite MDA can be performed rapidly and effectively by the use of short cation-exchange solid-phase extraction columns. Fig. 2 shows that MDMA and MDA are well resolved and separated from the endoge-

TABLE II

VALIDATION DATA FOR MDMA AND MDA

Parameter	MDMA	MDA
Linearity range (μg/ml)	0.5–17	0.08–1.6
Correlation coefficient	0.999	0.999
Mean recovery (%; n = 3):		
0.4 μg/ml sample	–	97.7
0.8 μg/ml sample	–	100.7
1.8 μg/ml sample	98.5	–
8.8 μg/ml sample	99.2	–
Mean precision (μg/ml; C.V., %; n = 6)		
0.4 μg/ml sample	–	0.39; 11.6
0.8 μg/ml sample	–	0.78; 6.1
1.8 μg/ml sampe	1.77; 0.9	–
8.8 μg/ml sample	8.69; 0.8	–
Detection limit[a]		
Absolute (ng)	2.8	1.3
Relative (μg/ml)	0.056	0.026

[a] Signal-to-noise ratio = 5:1.

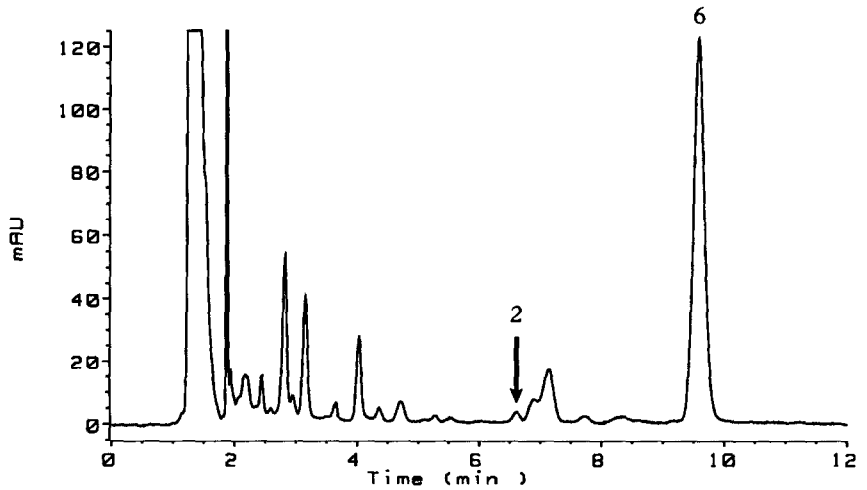


Fig. 2. Chromatogram of a human urine sample obtained after oral administration of 1.7 mg/kg MDMA. For peaks, see Table I.

nous matrix. The results of the recovery study for MDMA and MDA, listed in Table II with further validation data, show the efficiency of the extraction procedure. The volatility of MDMA and MDA may cause sample loss during the concentration step. This can be avoided by the addition of hydrochloric acid to the eluent and K_2HPO_4 to the extract before evaporation. After oral administration, MDMA is mainly exreted unchanged in urine. The concentrations found in the urine of four patients who received an oral dose of 1.7 mg/kg ranged from 1.48 to 5.05 μg/ml. The main metabolite identified was MDA, formed by N-demethylation and excreted in concentrations of 0.07 to 0.90 μg/ml. The pharmacokinetic data will be published in detail elsewhere.

Determination of mescaline in cactus plants

Solvent extraction was chosen for the extraction of cactus material. Mescaline is almost quantitatively extracted from the finely powdered and defatted cactus matrix by sonication with alkalized methanol (solvent/sample, 200:1, v/w). The selected I.S. (methoxamine) is a synthetic ring-substituted

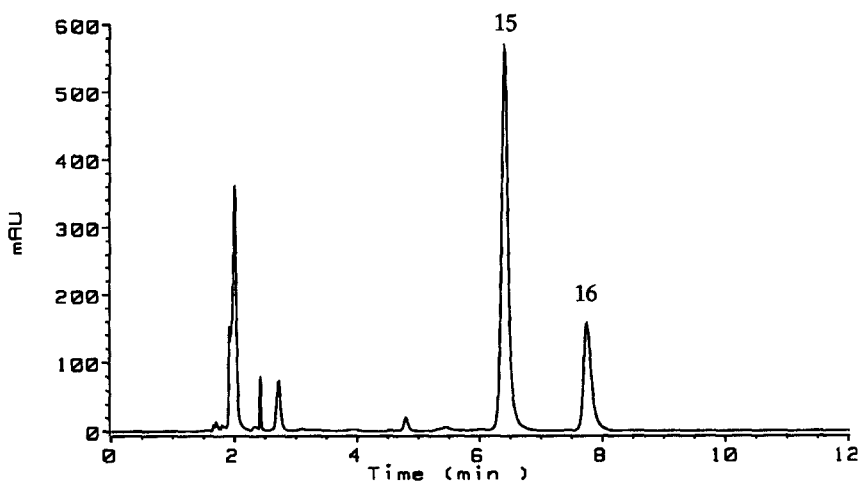


Fig. 3. Chromatogram of a *Trichocereus pachanoi* cactus specimen. For peaks, see Table I.

TABLE III

VALIDATION DATA FOR MESCALINE

Parameter	Mescaline
Linearity range (μg/ml)	20–75
Correlation coefficient	0.999
Mean recovery (%; n = 3)	99.1
Mean precision (μg/mg; C.V., %; n = 6)	9.9; 3.3
Detection limit[a]	
Absolute (ng)	1
Relative (μg/mg)	0.04

[a] Signal-to-noise ratio = 5.1.

phenylalkylamine derivative with chemical properties very similar to those of mescaline but not interfering chromatographically (Fig. 3). The efficiency of extraction was tested by spiking mescaline-free cactus material with mescaline hydrochloride. The recovery of mescaline (Table III) was >99%, showing that the loss of mescaline during the defatting process, which is necessary to remove interfering lipids and waxes, is not significant. The mescaline content of six *Trichocereus pachanoi* specimens ranged from 1.09 to 23.75 μg/mg dried cactus material, showing the extreme variability of the psychotropic potency.

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